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Simultaneous use of low methylesterified citrus pectin and EDTA as antioxidants in linseed/sunflower oil-in-water emulsions

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Abstract

In view of reducing synthetic antioxidants in emulsion-based food products, such as ethylene diamine tetraacetic acid (EDTA), this study aimed to investigate the simultaneous use of low methylesterified citrus pectin and EDTA to inhibit lipid oxidation as a consequence of their capacities to chelate Fe^{2+} . EDTA was found to be more effective in reducing lipid oxidation in oil-in-water (o/w) emulsions compared to pectin, probably due to its higher and stronger Fe^{2+} binding capacity. Moreover, pectin induced emulsion physical instability by depletion or bridging flocculation for 0.1% (w/v) pectin, or coalescence for 1% (w/v) pectin. A combination of low methylesterified pectin and EDTA in the o/w emulsions revealed a reduction of the antioxidant capacity of the individual compounds. In other words, no cooperative effect between pectin and EDTA to reduce the lipid oxidation was observed in this study. Although the reduction of EDTA concentrations in emulsions seems challenging, it is advised to conduct more fundamental research on pectin-EDTA interactions in model and real food systems.

Keywords

Citrus pectin; oxidative stability; physical stability; linseed/sunflower oil-in-water emulsion; EDTA; cation binding capacity

1. Introduction

Nowadays, decreasing the concentrations of artificial antioxidants in lipid-rich food products, particularly oil-in-water (o/w) emulsions containing poly-unsaturated fatty acids, is of high interest for food industries as well as for consumers. For instance, ethylene diamine tetraacetic acid (EDTA) is a widely used commercial antioxidant, mostly added to o/w emulsions such as mayonnaise or dressings (Ghorbani Gorji, Smyth, Sharma, & Fitzgerald, 2016; Jacobsen, Let, Nielsen, & Meyer, 2008). EDTA can chelate Fe^{2+} in the aqueous phase of such emulsions, hence remove Fe^{2+} from the vicinity of the oil droplet, preventing the

catalysis of the autoxidation of (polyunsaturated) fatty acids (Jacobsen et al., 2008; Mei, Decker, & McClements, 1998). In this sense, a ratio EDTA:Fe²⁺ of higher than one, i.e. excess of EDTA, has been shown to effectively reduce the Fe²⁺-mediated lipid oxidation (Hu, McClements, & Decker, 2004; Mozuraityte, Kristinova, Rustad, & Storrø, 2016). However, this synthetic chelating agent is reported to be associated with cytotoxic and genotoxic effects in cosmetic products (Lanigan & Yamarik, 2002). As a consequence, a limited use in foods would be preferable as a precaution, which in turn is also more appealing to consumers. Regarding the current industrial challenges to replace or reduce synthetic additives in foods, several natural anionic polysaccharides with cation chelating capacities such as pectin, xanthan, or alginate have been studied for their potential to increase the oxidative stability of food emulsions (Celus et al., 2018b; Chen, McClements, & Decker, 2010; Qiu, Zhao, Decker, & McClements, 2015; Salvia-Trujillo, Decker, & McClements, 2016; Shimada, Okada, Matsuo, & Yoshioka, 1996). It should be noted that addition of such natural polysaccharides can undesirably affect the viscosity of the food, while EDTA is assumed to have a negligible effect on the product flow characteristics. In the specific case of pectin, its antioxidant capacity can be mainly attributed to the non-methylesterified galacturonic acid (GalA) units in the linear subdomain of pectin, being homogalacturonan. The carboxyl groups of these GalA units are negatively charged at pH values above the *pKa* of pectin (2.8-4.1), enabling the interaction with divalent cations such as Fe²⁺ and partition them away from the oil-water interface in o/w emulsions. The percentage of methylesterified GalA units within the pectin chain is referred to as the degree of methylesterification (DM). Low DM pectin has been shown to exhibit a higher Fe²⁺ binding capacity (Celus et al., 2017) and therefore also a higher antioxidant capacity compared to high DM pectin in o/w emulsions (Celus et al., 2018b; Chen et al., 2010). Although the antioxidant capacity of pectin has not yet been largely explored and applied to foods, pectin is already widely used as a stabilizer or thickening agent

in food emulsions due to its ability to increase the viscosity of the continuous phase in o/w emulsions (Dickinson, 2003). However, at pectin concentrations above the *critical flocculation concentration* in the aqueous phase of emulsions, stabilized by previously adsorbed surfactants, physical instability might occur due to depletion or bridging flocculation (Dickinson, 2003).

Besides the use of pectin as a functional ingredient in food products, it can be *in situ* present as part of the raw material used in vegetable-based food emulsion production. Since demethylesterified citrus pectin has been shown to exhibit an antioxidant capacity, its (*in situ*) presence in food emulsions could contribute to a dual functionality, being a stabilizer and antioxidant. As a consequence, the concentrations of EDTA used in such food emulsions, which contain pectin, can possibly be decreased if a cooperative antioxidant capacity between pectin and EDTA exists. Indeed, it can be hypothesized that due to the additional Fe^{2+} binding capacity of pectin, less EDTA would be required to chelate all Fe^{2+} present and as such inhibit the lipid oxidation. This study therefore aims first to compare the antioxidant capacity of pectin with EDTA and secondly to explore the simultaneous use of EDTA and low methylesterified pectin as antioxidants in o/w emulsions.

2. Material and methods

2.1. Materials

High methylesterified citrus pectin (DM = 95%) was obtained from Sigma-Aldrich Belgium and used for the production of a pectin sample with a low degree of methylesterification. A blend of linseed/sunflower oil (30/70%) was kindly donated by Vandemoortele (Izegem, Belgium) and was used to study the lipid oxidation as it contained a high amount of polyunsaturated fatty acids. The fatty acid composition of the oil is shown in Table A (Supplementary material) and contained additionally 900 ppm natural tocopherols. FeSO_4 , FeCl_3 , BaCl_2 , Tween 80, iso-octane, methanol, ammonium thiocyanate, hexanal, 2-pentenal,

2-heptenal, 2-octenal, and 1-octen-3-ol were obtained from Sigma-Aldrich Belgium. 2-propanol was purchased from VWR Chemicals (Leuven, Belgium). Ultrapure water (organic free, 18.2 M Ω cm resistance) was supplied by a SimplicityTM water purification system (Millipore, Billerica, USA) and used for emulsion and sample preparation as well as for analytical experiments. All chemicals used were of analytical grade.

2.2. Pectin sample preparation and characterization

Low DM pectin was produced starting from a high methylesterified, linear citrus pectin with a DM of $95.1 \pm 1.8\%$ (CP95) by alkaline demethylesterification as described in our previous study (Celus et al., 2017). In brief, this alkaline demethylesterification was achieved by automatic titration of 0.1 M NaOH in order to maintain a pH of 11. The experiment was performed at 4 °C to exclude undesired pectin depolymerization. After addition of a predetermined amount of NaOH, the reaction was stopped by lowering the pH of the pectin solution to 4.5. The pH of the alkaline demethylesterified pectin sample was subsequently adjusted to pH 6, followed by dialysis (Spectra/Por[®], MWCO = 12-14 kDa) for 48 h against demineralized water and further lyophilization.

The modified pectin sample was characterized as described previously by Celus et al. (2017) for its (i) degree of methylesterification (DM) using Fourier transform infrared spectroscopy, (ii) galacturonic acid content by UV-Vis spectrophotometry, (iii) molar mass distribution using high performance size exclusion chromatography, coupled to multi-angle laser light scattering and refractive index and (iv) intrinsic cation concentration (⁴⁴Ca, ⁵⁶Fe and ⁶⁶Zn) by inductively coupled plasma mass spectrometry (ICP-MS).

2.3. Determination of the Fe²⁺ adsorption isotherm for low DM pectin

The Fe²⁺ adsorption isotherm of the alkaline demethylesterified pectin sample was determined according to the spectrophotometric method as previously described by Celus et al. (2017).

Briefly, 10 mL of deaerated pectin solutions (0.1% w/v) were enriched with 0 to 3.6 mM Fe^{2+} ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), transferred to previously rinsed dialysis membranes (Spectra/Por[®], MWCO = 3.5 kDa), and subsequently equilibrated at 15 °C for 48 h in closed jars containing 50 mL deaerated ultrapure water. In addition to the use of deaerated water, the experiment was performed in a nitrogen atmosphere using a pyramid portable glove bag (Erlab, Val de Reuil, France) to avoid an oxygen-rich atmosphere, hence Fe^{2+} oxidation. After equilibration between the non-bound Fe^{2+} outside and inside the membrane, the Fe^{2+} concentration of the outside solution was determined spectrophotometrically at 562 nm (1800 UV spectrophotometer, Shimadzu, Kyoto, Japan) after addition of 100 μL 0.01 M ferrozine (3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-*p,p'*-disulfonic acid monosodium salt hydrate) and 50 μL of 5 M ammonium acetate buffer (pH 9.5). The analysis was performed in triplicate and the Fe^{2+} adsorption capacity (q_e) of pectin was calculated based on the difference between the initial Fe^{2+} concentration and the concentration measured after equilibrium (Khotimchenko, Kolenchenko, & Khotimchenko, 2008):

$$q_e = \frac{[(C_0 \cdot V_{in} - C_e \cdot (V_{in} + V_{out}))]}{n_{\text{GalA}}}$$

with C_0 , the initial Fe^{2+} concentration (mM), C_e , the Fe^{2+} concentration at equilibrium (mM), V_{in} and V_{out} , the volume inside (0.01 L) and outside (0.05 L) the dialysis membrane, respectively, and n_{GalA} represents the absolute amount of GalA units in the pectin sample (mol). q_e is the equilibrium binding capacity of the pectin sample, expressed as mol Fe^{2+} /mol GalA. Taking into account the DM of the pectin sample, q_e can be expressed as mol Fe^{2+} /mol COO^- as well.

Since the adsorption equilibrium studies were performed at a constant temperature, adsorption isotherms indicating the amount of Fe^{2+} bound to each GalA or COO^- unit (q_e) as a function of the Fe^{2+} concentration at equilibrium (C_e) were obtained. The adsorption isotherms of the

pectin samples were subsequently modeled by the Langmuir adsorption model (Ho et al., 2002; Khotimchenko et al., 2008; Zhao et al., 2013). The Langmuir isotherm, which is the most widely used adsorption isotherm, assumes monolayer adsorption at homogenous and finite binding sites within the pectin chain (Foo & Hameed, 2010):

$$q_e = \frac{q_{\max} \cdot K_L \cdot C_e}{1 + K_L \cdot C_e}$$

with $q_{\max}$, the maximum binding capacity at the monolayer (mol Fe^{2+} /mol GalA or mol Fe^{2+} /mol COO^-), q_e , the binding capacity of a GalA or COO^- unit (mol Fe^{2+} /mol GalA or mol Fe^{2+} /mol COO^-) belonging to an Fe^{2+} equilibrium concentration, K_L , the Langmuir constant ($\text{L}/\text{mmol Fe}^{2+}$), which is a measure of the interaction energy and C_e , the Fe^{2+} equilibrium concentration ($\text{mmol Fe}^{2+}/\text{L}$).

Modeling of the experimental data of the pectin samples by the Langmuir adsorption isotherm was performed using non-linear one-step regression (SAS version 9.4, Cary, NC, USA).

2.4. Emulsion preparation

First, a coarse o/w emulsion was prepared by blending 20% (w/v) linseed/sunflower oil (LSSO), 2% (w/v) Tween 80 and 78% (w/v) of ultrapure water at 4000 rpm for 5 min using a high shear mixer (Silverson L5M-A, Silverson Machines, Massachusetts, USA). This o/w emulsion was further stabilized by high-pressure homogenization at 100 MPa for one pass (Pressure Cell Homogenizer, Stansted Fluid Power LTD., UK) and is referred to as the stock emulsion. The stock emulsion was subsequently diluted with a stock pectin solution of 1.33% (w/v) (prepared overnight to ensure complete dissolution, pH is around 6) and/or stock EDTA solutions of 1 mM, by stirring for 10 min. A final concentration of 5% (w/v) LSSO, 0.5% (w/v) Tween 80 and different pectin (0.1% and 1% w/v) and/or EDTA concentrations (0.05, 0.10 and 0.20 mM) were obtained. In the emulsion without pectin and EDTA, the stock emulsion was mixed with equal amounts of ultrapure water. In order to accelerate the lipid

oxidation, a stock Fe^{2+} solution of 0.5 M was freshly prepared by dissolving $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ into ultrapure water and added to the emulsions to obtain a final concentration of 0.10 mM Fe^{2+} (based on preliminary experiments, results not shown). Ferrous iron is chosen as an accelerator of the lipid oxidation, since the oxidized form (Fe^{2+}) has been reported to accelerate the oxidation reaction much faster than the reduced form (Fe^{3+}) (Choe & Min, 2006; Schaich, Shahidi, Zhong, & Eskin, 2013). The pH values of the emulsions were not adjusted in order to avoid the introduction of monovalent ions, possibly affecting the Fe^{2+} binding capacity of pectin or EDTA. However, the pH values were recorded during storage and varied between 4 and 6, being above the pKa of pectin and above at least two pKa values of EDTA (Mozuraityte et al., 2016), allowing the dissociation of carboxyl groups of pectin and EDTA.

A 0.1% pectin concentration was chosen as some polysaccharides have been reported to exhibit a significant antioxidant capacity at this concentration (Chen et al., 2010; Salvia-Trujillo et al., 2016), while concentrations higher than 1% could increase the emulsion viscosity substantially thus possibly affecting the lipid oxidation. The EDTA concentrations were chosen in order to obtain molar ratios EDTA: Fe^{2+} of 1:2, 1:1, and 2:1. EDTA has been shown to reduce effectively the Fe^{2+} -mediated lipid oxidation when present in ratios higher than 1:1.

The emulsions were quiescently stored (approximately 50 mL with little headspace volume) in triplicate in the dark at 35 °C for at least 21 days and both the oxidative (primary oxidation products, Section 2.6.1) as well as physical stability (Section 2.5) were evaluated. All the emulsions were manually agitated before measurements, except for the creaming index measurements, for which the emulsions were divided into separate small 10-mL glass tubes containing 5 mL aliquots so that they remained undisturbed. For the assessment of secondary oxidation products, freshly prepared emulsions (4 mL) were divided into 10-mL amber glass

vials (VWR International, Leuven, Belgium), tightly closed using screw-caps with silicon septum seals (Grace, Columbia, MD) and transferred to -80 °C after 0, 3, 7, 14, 16, 21, 28, 35, and 42 days of quiescent storage at 35 °C (in triplicate). At 35 °C the lipid oxidation reaction will be accelerated without inducing other chemical degradation reaction.

2.5. Evaluation of physical stability

The physical stability of the emulsions was determined at day 0, day 7, and day 14 of storage by measuring the particle electrical charge, creaming index, particle size distribution, and the microstructure as described in our previous study (Celus et al., 2018b). Briefly, the electrical charge (ζ -potential) of the oil droplets in the emulsions was determined by phase-analysis light scattering using automated capillary electrophoresis equipment (Zetasizer NanoZS, Malvern Instruments, Worcestershire, UK). The creaming index (%) of the emulsions was calculated by the ratio of the height of the cream layer (H_c) to the total height of the emulsion (H_e) using a caliper (Mitutoyo Belgium, Kruibeke, Belgium) (Guerra-Rosas, Morales-Castro, Ochoa-Martínez, Salvia-Trujillo, & Martín-Belloso, 2016). The particle size distributions of the emulsions were measured using laser diffraction equipment (Beckman Coulter Inc., LS 13 320 Miami, FL, USA). The particle sizes of the emulsions are reported as the d_{43} value (μm), which defines the volume-weighted mean particle size. Lastly, the microstructures of the emulsions were visualized at different magnifications with an optical microscope (Olympus BX41, Olympus Corporation, Tokyo, Japan) equipped with a digital camera (Olympus BX51, Olympus Optical Co. LTD., Tokyo, Japan).

2.6. Evaluation of lipid oxidative stability

2.6.1. Primary oxidation products

The formation of primary oxidation products in the emulsions was studied as a function of storage time by assessing the formation of peroxides spectrophotometrically as described in our previous study (Celus et al., 2018b). The oxidative stability was measured more

frequently than the physical stability (see Section 2.5), as the effect of pectin and/or EDTA on the lipid oxidation was the main focus in the present study. In brief, a 0.3 mL aliquot of the agitated emulsion was added to 1.5 mL iso-octane/2-propanol (3:1 v/v) in glass tubes and vortexed three times for 10 s. The tubes were subsequently centrifuged (Sigma 4-16KS centrifuge, swing-out rotor, Osterode am Harz, Germany) at 1000 g for 2 min at 20 °C. A mixture of the upper organic layer (0.2 mL), methanol/2-propanol (2:1 v/v) solution (2.8 mL), 3.94 M ammonium thiocyanate solution (15 µL) and 0.0072 M Fe^{2+} (15 µL) was measured spectrophotometrically for the Fe^{3+} concentration at 510 nm (Ultrospec 2100 Pro, Amersham Bioscience, Little Chalfont, UK) after 20 min in absence of light. The peroxide concentration (mEq peroxide/L emulsion) was calculated by dividing the concentration of Fe^{3+} in the emulsion (µg/mL) by 55.845 g/mol, the atomic weight of Fe^{3+} , and by two to express the peroxide value as mEq of peroxide instead of mEq of oxygen as explained by Shantha et al. (1994).

2.6.2. Secondary oxidation products

The formation of secondary oxidation products in the emulsions was determined as a function of storage time by analysis of volatile compounds. The volatiles were measured using headspace solid-phase microextraction coupled to gas chromatography-mass spectrometry (HS-SPME–GC–MS) as described by Palmers, Grauwet, Kebede, Hendrickx, and Van Loey (2014) with some modifications. Five volatile compounds were selected, being hexanal, 2-pentenal, 2-octenal, 1-octen-3-ol, and 2-heptenal. The former four compounds are reported to be secondary oxidation products of the autoxidation of linoleic acid, while 2-heptenal is an oxidation product of linolenic acid (Choe & Min, 2006). Linoleic and linolenic acid are the two predominant fatty acids present in the linseed/sunflower oil used (47.1 and 15.2%, respectively). In addition, these compounds exhibit peaks with the highest abundance as can be seen in the chromatogram in **Fig. A** (Supplementary material). Before analysis of the

secondary oxidation products, the samples were thawed overnight in an ice bath at 4 °C. Via the septum of the caps, 100 µL of a work solution (ca. 33 µg/mL ultrapure water) of internal standard (3-heptanone) was added to the emulsions using chilled gastight syringes (Hamilton, Bonaduz, Switzerland). Ultrapure water was subsequently added to obtain a standardized total volume of 4.6 mL. The exact amounts of solutions added were determined by differential weighing. The samples were homogenized and transferred to the cooling tray of the autosampler which was maintained at 5 °C.

The volatile compounds formed were analyzed using a GC system (GC system 7890A, Agilent Technologies, Diegem, Belgium) coupled to a mass spectrometer (MS) (5975C VL MSD, Agilent Technologies, Diegem, Belgium) and equipped with a CTC Combi PAL autosampler (CTC Analytics, Zwingen, Switzerland). Samples were incubated at 40 °C for 20 min under agitation at 500 rpm and volatile compounds were extracted at 40 °C for 10 min using an HS-SPME fiber coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (StableFlex, Supelco Inc., Bellefonte, PA, USA). After headspace extraction, the SPME fiber was inserted into the GC injection port, where the volatile compounds were desorbed for 2 min at 230 °C and injected onto the GC column in split mode (1/20). Chromatographic separation of the compounds was carried out on an HP-INNOWAX capillary column (60 m x 0.25 mm i.d., 0.25 µm film thickness, Agilent Technologies, Diegem, Belgium) with helium as carrier gas at a constant flow of 1.1 mL/min. The GC oven temperature was programmed at a starting temperature of 40 °C, which was maintained for 2 min, after which it was increased to 100 °C at 3 °C/min, then to 172 °C at 4 °C/min and subsequently ramped to 250 °C at 30 °C/min. After 2 min at 250 °C, the oven was cooled again to 40 °C. Mass spectra were obtained by electron ionization (EI) at 70 eV in combined SIM and scan mode (m/z 35–400). MS ion source and quadrupole temperatures were 230 °C and 150 °C, respectively. The selected qualifier and quantifier ions of the volatile compounds

and their retention times are presented in **Table 1**. Identification of the compounds was achieved by calculating the ratio of peak areas and retention times of qualifier ions to quantifier ions. Quantification of the volatile compounds was done using a calibration curve, which was prepared by spiking (0-500 μ L) freshly prepared emulsions with a work solution containing all of the selected compounds and 3-heptanone as internal standard. The concentration range of the calibration curves for each compound is also presented in **Table 1**.

2.7. Statistical analysis and data modeling

Statistical analysis of the experimental data was carried out with the statistical software JMP (JMP 14, SAS Institute Inc., Cary, NC, USA). A one-way analysis of variance (ANOVA) and a Tukey HSD test were carried out to analyze significant differences with a 95% confidence interval ($p < 0.05$) between the experimental data.

3. Results and discussion

3.1. Pectin sample characterization

Low methylesterified pectin was prepared by alkaline demethylesterification of commercial high methylesterified citrus pectin (CP95). The demethylesterified sample exhibited a DM of 10% and will be further noted as C10. The DM obtained and other characterization parameters (weight average molar mass, galacturonic acid content, and intrinsic iron content) of C10 and the commercial CP95 are shown in **Table 2** and are similar to the results reported in our previous study (Celus, Lombardo, Kyomugasho, Thielemans, & Hendrickx, 2018a). The DM significantly decreased from 95 to 10%, implying a difference in charge density and thus in the cation binding capacity. A decrease in molar mass was observed as well between CP95 and C10, indicating that depolymerization occurred to some extent during the alkaline demethylesterification reaction. However, this difference in molar mass was generally not considered to clearly influence the cation binding capacity of the pectin sample. The difference in GalA content and intrinsic Fe (sum of Fe^{2+} and Fe^{3+}) content between CP95 and

C10 were also considered to minimally affect the cation binding capacity, as previously reported (Celus et al., 2018a). Moreover, the intrinsic Fe content of C10 was 14 times lower than the Fe^{2+} content added to the emulsions to accelerate the lipid oxidation (0.10 mM), thus no contribution of the intrinsic Fe content is expected with regard to the lipid oxidation, neither for the adsorption experiment. To conclude, the alkaline demethylesterification resulted in a pectin sample which mainly differed in DM from the commercial high methylesterified citrus pectin, without compromising other structural properties.

3.2. Fe^{2+} adsorption isotherm for the demethylesterified citrus pectin sample

The Fe^{2+} adsorption capacity of the low methylesterified citrus pectin sample (C10) was determined by generation of an adsorption isotherm, which is presented in **Figure 1**. As explained in our previous study (Celus et al., 2017), the experimentally determined Fe^{2+} adsorption isotherm of pectin could be fitted by the Langmuir adsorption model, resulting in two main binding parameters: the maximum Fe^{2+} binding capacity (q_{max}), representing the plateau, and the interaction energy (K_L), as a measure for the steepness of the ascending part of the isotherm. The maximum binding capacity for C10 was estimated at 0.43 ± 0.01 mol Fe^{2+} /mol GalA or 0.48 ± 0.01 mol Fe^{2+} /mol COO^- , while the interaction energy was estimated at 410.9 ± 72.4 L/mmol Fe^{2+} . The isotherm and the associated estimated binding parameters obtained for C10 are in line with the adsorption isotherms of previously alkaline demethylesterified citrus pectin samples as reported by Celus et al. (2017). Indeed, our previous study revealed increasing q_{max} and K_L values with decreasing DM and the estimated values of C10 are higher than those of C20, being 0.427 mol Fe^{2+} /mol GalA and 179.6 L/mmol Fe^{2+} , respectively.

3.3. Emulsions physical stability

3.3.1. Particle electrical charge

The particle electrical charges of the emulsions with pectin and/or EDTA were measured by means of ζ -potential measurements. The ζ -potential values could indicate the adsorption of species at the oil-water interface and thus support the understanding of possible destabilization phenomena. The electrical charges (ζ -potential) of all emulsions were assessed at day 0, 7, and 14 of storage and are presented in **Table 3**.

Immediate determination of the ζ -potential, i.e. at day 0 of storage, revealed negatively charged oil droplets for all emulsions, which is in accordance to the results of our previous study (Celus et al., 2018b). Verkempinck et al. (2018) observed similar negative ζ -potential values for olive o/w emulsions stabilized by Tween80 (and pectin) at a pH of 7, which is comparable to the pH values of the emulsions in this study (approximately 5-6). Despite the use of a non-ionic surfactant, Tween 80, the negatively ζ -potential values could be explained by the presence of trace amounts of free fatty acids or the orientation of OH^- ions in the aqueous phase near the oil droplets, as mentioned in our previous study (Celus et al., 2018b). Although significant differences ($p \leq 0.05$) in ζ -potential values were found between the different emulsions, with values varying between -33.4 ± 1.0 and -46.3 ± 0.2 mV, the ζ -potential values were assumed to be similar for all emulsions at day 0 of storage. Thus, no clear effect of pectin or EDTA on the emulsion electrical charge can be found immediately after preparation of the emulsions.

During storage, the emulsion without pectin and EDTA exhibited comparable ζ -potentials, with -36.2 ± 1.0 mV at day 0 and -34.3 ± 0.7 mV at day 14 of storage, implying that no substantial change in electrical charge on the oil droplet surface occurred during storage. The addition of pectin, with or without EDTA, resulted for the majority of the emulsions in slightly more negative ζ -potentials during storage compared to day 0, which can be attributed

to the negative charges of the low methylesterified pectin sample. The emulsions with 1% C10 or 1% C10 + 0.05 mM EDTA showed for instance a decrease in ζ -potential from -36.1 ± 0.7 to -50.0 ± 1.5 mV or from -36.5 ± 2.9 to -58.4 ± 1.2 mV, respectively. Our results are in accordance to the results of Surh et al. (2006), who reported more negative ζ -potential values for sodium caseinate-stabilized corn (5 wt%) o/w emulsions with low methylesterified citrus pectin (0.25 wt%) at a pH between 5 and 6, compared to such emulsions without pectin. The more negative ζ -potential values for emulsions with pectin observed in the present study, could, on the one hand, indicate that pectin is situated in the slipping plane surrounding the oil droplets in the emulsion, as this is the place where the zeta potential is measured (Clogston & Patri, 2011). On the other hand, according to Surh et al. (2006), strong electrostatic repulsion between the negatively charged oil droplets and negatively charged pectin samples could imply the presence of pectin in the aqueous phase of the emulsions. As multiple and contrasting hypothesizes are possible, the location of pectin in the emulsion, being in the aqueous phase or near the oil droplet phase, cannot be clearly defined by these results.

In contrast to pectin addition, the addition of EDTA only to the emulsions did not clearly affect the ζ -potential during storage. For instance, the ζ -potentials of the emulsions with 0.05, and 0.20 mM EDTA were -34.7 ± 1.0 and -40.9 ± 0.6 mV at day 14, respectively, compared to -33.4 ± 1.0 and -36.0 ± 0.9 mV, respectively, at day 0. The fact that the ζ -potential values of the emulsions with EDTA only did not change substantially, could be attributed to the lower number of negatively chargeable carboxyl groups introduced in the emulsions compared to pectin. For EDTA, 0.2, 0.4, or 0.8 mM COOH is present in the emulsions with 0.05, 0.10, or 0.20 mM EDTA, respectively (1 mol of EDTA contains 4 mol of COOH groups), while 3.9 or 39 mM COOH for 0.1 and 1% (w/v) C10, respectively (calculated based on the pectin concentrations, the GalA content in the pectin samples, and the number of non-methylesterified GalA units). In this sense, the contribution of EDTA to the ζ -potential in the

emulsions containing a combination of pectin and EDTA could be assumed as negligible, thus mainly pectin influences the electrical charge of those emulsions.

3.3.2. *Emulsion particle size, creaming index, and microstructure*

In order to determine possible (de)stabilization phenomena, due to the addition of pectin or EDTA to the o/w emulsions, the particle size distribution, creaming index, and microstructure of the emulsions were assessed during storage. **Table 3** represents the volume-weighted mean diameter (d_{43}) for each emulsion as a function of storage time (day 0, 7, and 14), together with the creaming index (%) measured at day 14 of storage. The representative micrographs of each emulsion, captured at day 0, 7, and 14 of storage are shown in **Figure 2, 3, and 4**.

The emulsion without pectin or EDTA exhibited a mean particle size of $1.04 \pm 0.04 \mu\text{m}$, which slightly increased during storage (**Table 3**). However, this increase in particle charge is not considered to impact the emulsion stability, as the emulsions remained stable during storage, which is evidenced by the absence of a cream layer after 14 days (**Table 3**) and by the microscopic images (**Figure 2 a-c**). The emulsions with the addition of EDTA only were stable during storage as well, as can be derived from the mean particle size (**Table 3**), which is comparable over time, and the micrographs (**Figure 3 a-i**). Moreover, no creaming could be detected in these emulsions. Thus, EDTA addition, irrespective of the concentration, did not compromise the steric stabilization between the oil droplets provided by Tween 80. The addition of pectin only and combinations of pectin and EDTA to the emulsions did not immediately, i.e. at day 0, affect the mean particle size, with values varying between $1.07 \pm 0.11 \mu\text{m}$ and $2.98 \pm 0.02 \mu\text{m}$ (**Table 3**). Nevertheless, the micrographs in **Figure 2 (d and g)** and **Figure 4 (a, d, g, j, and h)** reveal some droplet flocculation at day 0, which was more pronounced in the emulsions with 1% (w/v) C10 compared to 0.1% (w/v) C10. These observations are in agreement with our previous study and could be attributed to depletion or bridging flocculation (Celus et al., 2018b). Since EDTA did not compromise the emulsion

stability at day 0, as explained before, it can be stated that the flocculation observed in the emulsions with pectin and EDTA is mainly attributed to pectin.

During storage, the mean particle size of the emulsions with 0.1% (w/v) C10, with or without EDTA, did not clearly change, while emulsions with 1% (w/v) C10, with or without EDTA, revealed a significant increase in particle size (**Table 3**). Despite the oil droplet sizes in the emulsions with 0.1% (w/v) pectin remained generally constant, being $1.25 \pm 0.03 \mu\text{m}$ (LSSO + 0.1% C10) and $2.07 \pm 0.11 \mu\text{m}$ (LSSO + 0.1% C10 + 0.05 mM EDTA) after 14 days of storage, a comparable cream layer could be detected as in the emulsions with 1% (w/v) pectin, as indicated by the creaming indices (**Table 3**). As reported in our previous study, this evidences that the flocs which are formed immediately after mixing in the emulsion with pectin result in gravitational separation and creaming of the oil droplets after 14 days of storage (Celus et al., 2018b). Moreover, since the oil droplet sizes increase for the emulsions with 1% (w/v) pectin, up until values varying between 4.56 ± 0.29 and $19.12 \pm 3.18 \mu\text{m}$ (**Table 3**), it indicates coalescence of the oil droplets during storage, which is also reflected visually by the larger oil droplets compared to 0.1% (w/v) C10 in **Figure 2i** and **Figure 4 f, i, l, and o**. Our previous study on the effect of pectin with varying DM on emulsion physical stability revealed similar conclusions, being 0.1% (w/v) pectin causing depletion or bridging flocculation, while 1% (w/v) pectin led to coalescence of the emulsions (Celus et al., 2018b).

3.4. Emulsions lipid oxidative stability

The influence of pectin and/or EDTA addition on the Fe^{2+} -catalyzed lipid oxidation in o/w emulsions was studied by determination of the primary (**Figure 5**) and secondary (**Figure 6**) oxidation products as a function of storage time. Primary oxidation products were monitored by means of peroxide concentration as a function of storage time. The formation of peroxides during the autocatalytic lipid oxidation process can theoretically be described by three consecutive reactions consisting firstly of a lag phase where the formation of free radicals

occurs (initiation) without a significant increase in peroxide concentration. Subsequently, lipid peroxides are formed during propagation, which is associated with a progressive increase in peroxides. As the lipid oxidation proceeds, peroxides decompose to secondary oxidation products (termination), resulting in a maximum level of peroxides after which the decomposition process dominates (Frankel, 1980; Manzocco, Calligaris, & Nicoli, 2010). Those three reactions were clearly observed during storage of the majority of the o/w emulsions with pectin only (**Figure 5a**), EDTA only (**Figure 5b**), and the pectin/EDTA combinations (**Figure 5c**). As peroxides are decomposed into secondary oxidation products, multiple volatiles are formed. In this study, the concentrations hexanal, 2-pentenal, 2-heptenal, 2-octenal, and 1-octen-3-ol are monitored as a function of storage time for all o/w emulsions. **Figure 6** represents the sum of these volatile concentrations for each time moment in order to gain insight into the overall formation of secondary oxidation products, as preliminary experiments showed that these five selected volatiles contribute to the highest percentages of all volatiles formed (results not shown). The amount of each volatile separately as a function of storage time is presented in **Figure B** in supplementary material for each emulsion and as can be seen, the profiles of each compound are very similar to the profile of the sum of the volatiles. From **Figure 6** it can be observed that the secondary oxidation products exhibit a steady increase (no immediate increase since the peroxides need to be decomposed first) after which no clear decrease is observed as they remain present in the sample once formed.

Addition of low methylesterified citrus pectin (C10) to the emulsion led to an overall decrease of peroxide formation and thus lipid oxidation, which was concentration dependent (**Figure 5a**). Although no effect on the lag phase was observed compared to the control, the decreased lipid oxidation is reflected by a slower increase in peroxide concentration and by a lower maximum peroxide value reached. For instance, the addition of 0.1% (w/v) C10 decreased the

maximum peroxide value from 22.6 to 16.8 mEq peroxide/L emulsion, while this value is further reduced with 40% to 10.1 mEq peroxide/L emulsion by increasing the concentration to 1% (w/v) C10. It should be noted that the maximum peroxide values in **Figure 5** cannot be evidenced. However, comparison of these maximum peroxide values of the different emulsions was found to be useful in this study in order to describe the effect of pectin and/or EDTA addition on the lipid oxidation. In contrast to the peroxide values, the concentration of secondary oxidation products formed in this study was not influenced by the addition of 0.1% (w/v) C10, neither by 1% (w/v) C10 (**Figure 6a**). Thus, despite pectin addition reduced the amount of peroxides compared to the emulsion without pectin or EDTA, similar concentrations of secondary oxidation products were found between the emulsions with pectin and the emulsion without pectin or EDTA. This result is remarkable for which no immediate explanation can be found. Comparison of the antioxidant capacity of C10 with previous research revealed a higher antioxidant capacity for P13 (enzymatically demethylesterified citrus pectin with DM of 13%) in terms of maximum peroxide value, both for 0.1 (w/v) and 1% (w/v) compared to C10, despite their comparable DMs (Celus et al., 2018b). For instance, the maximum peroxide values reached for P13 and C10 were 7.4 and 16.8 mEq peroxide/L emulsion, for 0.1% (w/v), whereas 2.5 and 10.1 mEq peroxide/L emulsion for 1% (w/v), respectively. The observation that the enzymatically demethylesterified pectin exhibited more reduced peroxide values compared to the chemically demethylesterified pectin with a similar DM is surprisingly. Previous work showed that pectin with a higher number or longer blocks of successive negatively charged GalA units (as a consequence of the enzymatic demethylesterification) can bind a higher amount or stronger to pro-oxidant metal ions. However, it could be assumed that at very low DM, pectin consists of an almost completely demethylesterified backbone, resulting in less clear differences in the distribution pattern between an enzymatic or chemical demethylesterified pectin sample.

Despite no clear difference in cation binding was observed in our previous study (Celus et al., 2017) and consequently no difference in antioxidant capacity could be expected between the low DM enzymatic and chemical demethylesterified pectin sample, the enzymatically demethylesterified pectin with low DM seem to be of more interest for its use as an antioxidant in o/w emulsions compared to alkaline demethylesterified pectin of comparable DM.

The lipid oxidation of emulsions containing 0.10 mM Fe^{2+} and 0.05, 0.10, or 0.20 mM EDTA was studied in order to compare the lipid antioxidant capacity of pectin with a commercially used antioxidant. Although the addition of 0.05 mM EDTA already showed some impact on the peroxide formation, a clear inhibition of the lipid oxidation in terms of peroxide value during the total storage period was observed in the emulsions with 0.10 mM and 0.20 mM EDTA (**Figure 5b**). Similarly, the formation of secondary oxidation products was slightly reduced by 0.05 mM EDTA, while a clear reduction was observed by the addition of 0.10 mM and 0.20 mM EDTA to the emulsions (**Figure 6b**). This result is in agreement with the fact that EDTA binds to Fe^{2+} in a 1:1 ratio (Hu et al., 2004; Mozuraityte et al., 2016), implying that all free Fe^{2+} was bound to EDTA and thus no substantial amount was available to catalyze the lipid oxidation. Despite the fact that emulsions with 0.10 mM and 0.20 mM EDTA contained a lower number of negatively chargeable carboxyl groups, being approximately 0.40 mM and 0.80 mM COO^- , respectively, compared to the emulsions with 1% C10 (the pectin concentration with the highest antioxidant capacity), being approximately 39 mM COO^- , the emulsions with 0.10 mM and 0.20 mM EDTA exhibited clearly lower peroxide values and volatiles. This indicated a much higher Fe^{2+} -binding capacity for EDTA compared to low DM citrus pectin, confirming the clearly higher lipid antioxidant capacity of EDTA. In addition, the binding constant of EDTA (approximately $4.9 \cdot 10^8 \text{ M}^{-1}$) (Andjelković et al., 2006) is far higher than observed for C10 in Section 3.2 (approximately 0.4 M^{-1}).

In view of replacing synthetic antioxidants by natural ones, the use of pectin as an alternative for EDTA will be challenging given that EDTA is more effective in chelating Fe^{2+} . Therefore, pectin and EDTA were simultaneously added to the emulsions in different concentrations to study the cooperative effect between the antioxidant capacity of those components, if any. In this sense, concentrations of EDTA in food products could possibly be reduced, without compromising the oxidative stability of such food products. **Figures 5c** and **6c** illustrate the peroxides value and secondary oxidation products of all pectin/EDTA combinations, respectively. A clear reduction in peroxides values was observed for the emulsions with 0.1% C10 + 0.05 mM EDTA, 1% C10 + 0.05 mM EDTA, and 1% C10 + 0.10 mM EDTA (**Figure 5c**), while no reduction in secondary oxidation products was observed for these samples compared to the control sample (**Figure 6c**). Moreover, no clear differences in both primary and secondary oxidation could be distinguished between these samples mutually. Especially for the emulsion with 1% C10 + 0.10 mM EDTA, this limited effect on the lipid oxidation is remarkable, as 1% C10 only (**Figure 5a**) and 0.10 mM EDTA only (**Figure 5b** and **6b**) proved to be effective in reducing the lipid oxidation. From **Figure 5c** and **Figure 6c**, it can be seen that the EDTA concentration should be raised to 0.20 mM in combination with 1% (w/v) pectin in order to clearly inhibit lipid oxidation. These results imply no cooperative effect between pectin and EDTA with regard to their antioxidant capacity. Moreover, it can be assumed that the antioxidant capacity of EDTA is reduced by the addition of pectin, since a concentration of 0.20 mM EDTA is needed in combination with pectin to substantially reduce the lipid oxidation products, while a concentration of 0.10 mM EDTA is sufficient in an emulsion with EDTA only to inhibit the lipid oxidation. Some possible explanations can be formulated to explain this remarkable observation. Firstly, EDTA could be bound to Ca^{2+} , which originates from the pectin sample. EDTA is thus less available to bind Fe^{2+} and subsequently reduce the lipid oxidation. As such, a higher EDTA concentration is required to

equally decrease the lipid oxidation. The Ca^{2+} concentration introduced in the emulsion via the pectin sample (1% w/v) would be approximately 0.25 mM (results not shown), which is 2.5-fold higher than the added 0.10 mM Fe^{2+} content, presuming a higher affinity of EDTA for Ca^{2+} compared to Fe^{2+} . Moreover, EDTA showed a higher binding constant for Ca^{2+} compared to pectin, explaining the transition of intrinsic Ca^{2+} from pectin to EDTA. Secondly, and of minor importance, the number of chargeable carboxyl groups in the pectin sample at 1% (w/v) is a 100-fold higher compared to the carboxyl groups in the emulsion with the 0.10 mM EDTA. The presence of carboxyl groups of EDTA could thus be negligible compared to pectin. Despite the higher and stronger binding capacity of EDTA compared to pectin, Fe^{2+} would then rather be bound to pectin, explaining the limited effect of the pectin-EDTA combinations on the lipid oxidation since it is not that strongly bound.

Conclusion

The simultaneous use of EDTA and low methylesterified citrus pectin as antioxidants in oil-in-water emulsions was studied in view of reducing the concentrations of the synthetic antioxidant EDTA in food products. The antioxidant capacity of the low methylesterified pectin was assumed to be due to chelation of Fe^{2+} via negatively charged carboxyl groups. The Fe^{2+} binding capacity of the chemically demethylesterified pectin sample was in agreement with the results of our previous study, being a higher Fe^{2+} binding capacity with decreasing DM. Despite a very low DM of 10% was obtained in this study, the Fe^{2+} binding capacity was substantially lower compared to EDTA. This was also reflected by the assessment of the lipid oxidation during storage. The emulsions containing EDTA only exhibited a much higher oxidative stability compared to the emulsions with pectin only. Moreover, pectin addition to emulsions induced physical instability of the emulsion due to depletion or bridging flocculation for 0.1% (w/v) pectin and coalescence for 1% (w/v) pectin. When both antioxidants were simultaneously added to the emulsions, a higher concentration

of EDTA (0.20 mM) was necessary in order to inhibit the lipid oxidation to the same extent as in the emulsion with EDTA only (0.10 mM). This implies that the high antioxidant capacity of EDTA was surprisingly reduced by pectin. This reduction could possibly be explained by the decrease in Fe^{2+} binding capacity of EDTA due to the chelation of Ca^{2+} , originating from the pectin sample. In conclusion of this study, no cooperative antioxidant effect was observed between pectin and EDTA. In order to reach the goals to decrease EDTA concentrations without compromising the oxidative stability of emulsion-based food products, more research is needed.

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Declaration of interests

none

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Compound	Retention time (min)	Quantifier ion	Qualifier ion	Range standard curve (µg/mL emulsion)
Hexanal	10.92	56	41	0-20
2-pentenal	12.75	55	83	0-5
3-heptanon (IS)	13.69	57	41	na.
2-heptenal	21.13	41	55	0-10

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Tables

Table 1: Overview of retention time, quantifier and qualifier ions of the five selected volatiles and internal standard (IS), measured by GC-MS. The range of the standard curves for the five volatiles is given as well.

2-octenal	25.56	41	55	0-30
1-octen-3-ol	26.29	57	43	0-5

Table 2: Overview of structural characteristics $\pm$ standard deviation of the commercial high methylesterified citrus pectin (CP95) and the alkaline demethylesterified pectin sample (C10). DM (%) is the degree of methylesterification, M_w (kDa) refers to the weight-average molar mass, and GalA content (g/gpectin) is the galacturonic acid content in the pectin sample. Different letters (a-b) indicate significant ($p \leq 0.05$) differences between the pectin samples for a particular structural characteristic.

Sample code	DM (%)	M_w (kDa)	GalA content (g/g pectin)	Intrinsic Fe content ($\mu\text{g/g}$ pectin)
CP95	95.1 ± 1.8^a	56.1 ± 1.3^a	0.69 ± 0.01^b	17.1 ± 1.0^a
C10	10.0 ± 1.4^b	38.7 ± 0.8^b	0.85 ± 0.03^a	39.9 ± 22.3^a

Table 3: ζ -potential values (mV) and volume-weighted mean particle sizes (d_{43} , μm) $\pm$ standard deviation as a function of storage time (day 0, day 7, and day 14) and creaming index (CI, %) at day 14 of storage of linseed/sunflower emulsions without (no pectin/no EDTA) or with EDTA and/or alkaline demethylesterified citrus pectin (C10). Different capital letters (A-C) indicate significant ($p \leq 0.05$) differences in ζ -potential values or d_{43} values between storage times of the same sample. Different lower case letters (a-g) indicate significant ($p \leq 0.05$) differences in ζ -potential values, d_{43} values or CI between different samples of the same storage time.

Emulsion	ζ -potential (mV)			d_{43} (μm)			CI (%)
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14	Day 14
No pectin/No EDTA	$-36.2 \pm 1.0^{\text{Bab}}$	$-36.2 \pm 1.0^{\text{Ba}}$	$-34.3 \pm 0.7^{\text{Aa}}$	$1.04 \pm 0.04^{\text{Cc}}$	$1.15 \pm 0.05^{\text{Bd}}$	$1.28 \pm 0.06^{\text{Ad}}$	0^{c}
0.05 mM EDTA	$-33.4 \pm 1.0^{\text{Aa}}$	$-33.5 \pm 0.8^{\text{Aa}}$	$-34.7 \pm 1.0^{\text{Aab}}$	$1.00 \pm 0.05^{\text{Cc}}$	$1.21 \pm 0.03^{\text{Bcd}}$	$1.49 \pm 0.15^{\text{Ad}}$	0^{c}
0.10 mM EDTA	$-41.5 \pm 1.4^{\text{Accd}}$	$-41.3 \pm 0.5^{\text{Ab}}$	$-38.2 \pm 3.3^{\text{Abc}}$	$1.32 \pm 0.02^{\text{Bb}}$	$1.35 \pm 0.01^{\text{ABbcd}}$	$1.41 \pm 0.08^{\text{Ad}}$	0^{c}
0.20 mM EDTA	$-36.0 \pm 0.9^{\text{Aab}}$	$-39.8 \pm 0.1^{\text{Bb}}$	$-40.9 \pm 0.6^{\text{Bc}}$	$0.99 \pm 0.05^{\text{Cc}}$	$1.15 \pm 0.04^{\text{Bd}}$	$1.26 \pm 0.02^{\text{Ad}}$	0^{c}
0.1% C10	$-45.7 \pm 1.6^{\text{Ae}}$	$-55.9 \pm 1.4^{\text{Bd}}$	$-54.1 \pm 1.4^{\text{Bf}}$	$1.09 \pm 0.11^{\text{Bc}}$	$1.23 \pm 0.04^{\text{Accd}}$	$1.25 \pm 0.03^{\text{Ad}}$	$17.7 \pm 1.9^{\text{a}}$
1% C10	$-36.1 \pm 0.7^{\text{Aab}}$	$-57.4 \pm 0.5^{\text{Cd}}$	$-50.0 \pm 1.5^{\text{Be}}$	$1.07 \pm 0.10^{\text{Cc}}$	$3.34 \pm 0.43^{\text{Ba}}$	$4.56 \pm 0.29^{\text{Accd}}$	$18.5 \pm 1.9^{\text{a}}$
0.1% C10 + 0.05 mM EDTA	$-46.3 \pm 0.2^{\text{Ae}}$	$-56.8 \pm 1.9^{\text{Bd}}$	$-58.8 \pm 0.8^{\text{Bg}}$	$1.35 \pm 0.02^{\text{Cb}}$	$3.28 \pm 0.48^{\text{Aa}}$	$2.07 \pm 0.11^{\text{Bd}}$	$18.2 \pm 0.7^{\text{a}}$
1% C10 + 0.05 mM EDTA	$-36.5 \pm 2.9^{\text{Aab}}$	$-61.8 \pm 2.2^{\text{Be}}$	$-58.4 \pm 1.2^{\text{Bg}}$	$1.42 \pm 0.05^{\text{Cb}}$	$3.33 \pm 0.09^{\text{Ba}}$	$12.62 \pm 0.39^{\text{Ab}}$	$18.8 \pm 1.1^{\text{a}}$
1% C10 + 0.10 mM EDTA	$-38.1 \pm 2.5^{\text{Abc}}$	$-56.8 \pm 0.5^{\text{Bd}}$	$-57.1 \pm 1.0^{\text{Bfg}}$	$1.40 \pm 0.04^{\text{Bb}}$	$2.94 \pm 0.09^{\text{Ba}}$	$19.12 \pm 3.18^{\text{Aa}}$	$20.1 \pm 0.4^{\text{a}}$
1% C10 + 0.20 mM EDTA	$-43.9 \pm 0.5^{\text{Ade}}$	$-51.4 \pm 1.5^{\text{Bc}}$	$-45.5 \pm 1.1^{\text{Ad}}$	$2.99 \pm 0.01^{\text{Ba}}$	$1.82 \pm 0.06^{\text{Bb}}$	$14.43 \pm 2.93^{\text{Ab}}$	$10.9 \pm 0.0^{\text{b}}$
1% C10 + 0.50 mM EDTA	$-41.0 \pm 1.2^{\text{Accd}}$	$-51.3 \pm 1.0^{\text{Cc}}$	$-45.2 \pm 1.3^{\text{Bd}}$	$2.98 \pm 0.02^{\text{Ba}}$	$1.68 \pm 0.01^{\text{Bbc}}$	$7.45 \pm 1.56^{\text{Ac}}$	$10.3 \pm 1.3^{\text{b}}$

Table A (supplementary material): Fatty acid composition of the linseed/sunflower oil (30/70%).

Fatty acid	Content (%)
C16:0	6.15
C18:0	4.15
C18:1	26.17
C18:2	47.1
C18:3	15.21
C20:0	0.13
C20:1	0.22
C22:0	0.59
C22:1	0.15

Figure captions

Figure 1: Adsorption isotherm representing the equilibrium cation binding capacity, q_e (mol Fe^{2+} /mol GalA) as a function of the cation equilibrium concentration, C_e (mmol/L) for Fe^{2+} binding to alkaline demethylesterified citrus pectin sample (C10). Symbols indicate the experimental data with standard deviation. Black curve is the modeled Langmuir adsorption isotherm.

Figure 2: Micrographs at day 0, 7, and 14 of storage of the linseed/sunflower oil (LSSO) emulsions without pectin and EDTA, and with single alkaline demethylesterified pectin (C10) (0.1% w/v or 1% w/v). Scale bar represents 50 μm .

Figure 3: Micrographs at day 0, 7, and 14 of storage of the linseed/sunflower oil (LSSO) emulsions with single EDTA (0.05 mM, 0.10 mM, or 0.20 mM). Scale bar represents 50 μm .

Figure 4: Micrographs at day 0, 7, and 14 of storage of the linseed/sunflower oil (LSSO) emulsions with combinations of pectin (0.1 or 1% w/v) and EDTA (0.05, 0.10, 0.20, or 0.50 mM). Scale bar represents 50 μm .

Figure 5: Peroxide concentration (mEq peroxide/L emulsion) with standard deviation as a function of storage time (days) of the linseed/sunflower oil (LSSO) emulsions A) with alkaline demethylesterified pectin only (C10), B) EDTA only, and C) a combination of alkaline demethylesterified pectin (C10) and EDTA. All LSSO emulsions contained 0.1 mM Fe^{2+} .

Figure 6: Concentration sum of volatile secondary oxidation products (hexanal, 2-pentenal, 2-heptenal, 2-octenal, and 1-octen-3-ol) (mg/L emulsion) with standard deviation as a function of storage time (days) of the linseed/sunflower oil (LSSO) emulsions with A) demethylesterified pectin only (C10), B) EDTA only, and C) a combination of alkaline demethylesterified pectin (C10) and EDTA. All LSSO emulsions contained 0.1 mM Fe²⁺.

Supplementary material

Figure A: Chromatogram of volatiles measured with GC-MS. 1 = hexanal, 2 = 2-pentenal, 3 = 2-heptenal, 4 = 2-octenal, and 5 = 1-octen-3-ol. IS is internal standard, 3-heptanon.

Figure B: Concentration volatile secondary oxidation products (hexanal, 2-pentenal, 2-heptenal, 2-octenal, and 1-octen-3-ol) (mg/L emulsion) with standard deviation as a function of storage time (days) of the linseed/sunflower oil (LSSO) emulsions with first column demethylesterified pectin only (C10), second column EDTA only, and third column a combination of alkaline demethylesterified pectin (C10) and EDTA. All LSSO emulsions contained 0.1 mM Fe²⁺.

—○— No pectin/No EDTA —●— 0.1% C10 —●— 1% C10

—○— No pectin/No EDTA —●— 0.05 mM EDTA
 —●— 0.10 mM EDTA - -○- 0.20 mM EDTA

—○— No pectin/No EDTA —●— 0.1% C10 + 0.05 mM EDTA
 —●— 1% C10 + 0.05 mM EDTA - -○- 1% C10 + 0.10 mM EDTA
 - -●- 1% C10 + 0.20 mM EDTA - -●- 1% C10 + 0.50 mM EDTA

Low methylesterified citrus pectin was produced by alkaline demethylesterification.

The Fe^{2+} binding capacity of the pectin sample was studied by an adsorption isotherm.

EDTA exhibited a higher antioxidant capacity compared to low methylesterified pectin.

Pectin (0.1 and 1% w/v) caused instability of linseed/sunflower oil emulsions.

No cooperative effect between pectin and EDTA to reduce lipid oxidation was found.

Declaration of interest: none

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